

Developmental Exposure to Polychlorinated Biphenyls (Aroclor 1254) Reduces Circulating Thyroid Hormone Concentrations and Causes Hearing Deficits in Rats¹

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Developmental hypothyroidism causes growth deficits, motor dysfunction, and hearing disorders in humans and animals. Therefore, environmental toxicants, such as polychlorinated biphenyls (PCBs), may secondarily affect these endpoints via thyrotoxicity. In this study, Long-Evans rats were given Aroclor 1254 (po), at 0, 1, 4, or 8 mg/kg from Gestation Day 6 through Postnatal Day (PND) 21. We evaluated the offspring at various age intervals for circulating thyroid hormone concentrations [thyroid-stimulating hormone, and free and total triiodothyronine (T3) and thyroxine (T4)], body weight, eye opening, survival, motor activity development, auditory startle response, and auditory thresholds. Circulating T4 concentrations were sharply reduced in a dose-dependent fashion in PCB-exposed groups at PND 1, 7, 14, 21, and 30 but recovered to control levels by PND 45. Moderate reductions in T3 concentrations were apparent in the 4 and 8 mg/kg groups on PND 21 and 30. Deficits in body weight gain and early eye-opening were apparent in the treated pups; by weaning, pup mortality was 20% in the 4 mg/kg group and 50% at the highest dose. Motor activity was also transiently reduced in 15 day old offspring from the 8 mg/kg group. At this dose, animals showed reduced auditory startle amplitudes at PND 24, but not when tested as adults. Importantly, Aroclor 1254 caused permanent auditory deficits (20-30 dB threshold shift) at the lowest frequency tested (1 kHz) in both the 4 and 8 mg/kg groups, whereas auditory thresholds were not significantly affected at higher frequencies (4, 16, 32, or 40 kHz). These data indicate that while some effects of Aroclor 1254

exposure are dissimilar to drug-induced hypothyroidism (e.g., age of eye opening), effects on hormone levels and body weight are comparable. Detection of auditory deficits in PCB-treated animals is a novel finding and may reflect the effects of thyroid hormone disruption on the development of the cochlea. © 1995 Academic Press, Inc.

There is growing concern that a number of synthetic chemical pollutants may affect the health of humans and wildlife via disruption of endocrine functions (Colborn and Clement, 1992; Colborn *et al.*, 1993). For instance, polychlorinated biphenyls (PCBs) are ubiquitous, environmentally persistent, and bioaccumulative agents (see Safe, 1994 for review) that have been shown to affect a number of endocrine targets (McKinney and Waller, 1994). While the most recent attention has focused on the potential estrogenic effects of PCBs and related compounds (e.g., Colborn *et al.*, 1993), it is likely that PCB-induced disruption of thyroid function may contribute to their toxicological effects. The thyrotoxic effects of PCBs are thought to be related to the structural similarities shared between these compounds and thyroid hormones (McKinney, 1989; Rickenbacher *et al.*, 1986). Hence, the PCB-induced reduction in circulating thyroxine (T4) in adult (Barter and Klaassen, 1992; Byrne *et al.*, 1987; van den Berg *et al.*, 1988) and developing organisms (Collins and Capen, 1980; Morse *et al.*, 1993; Morse and Brouwer, 1994; Ness *et al.*, 1993) has been attributed to increased excretion of free T4 due to competitive binding of PCBs with thyroid hormone transport proteins (Rickenbacher *et al.*, 1986; Brouwer and Van den Berg, 1986), direct damage to the thyroid gland (Ness *et al.*, 1993), and/or amplified biliary excretion of T4 due to induction of UDP-glucuronosyltransferase (Barter and Klaassen, 1992). Thus, it has been suggested that the adverse effects of PCBs on the developing nervous system (see Tilson *et al.*, 1990) may be linked to their thyrotoxic effects (Porterfield, 1994).

Hypothyroidism, which occurs during the period of neurological development in humans and laboratory animals, pro-

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duces a number of well-characterized effects (Porterfield, 1994). In humans, dietary iodine deficiency during pregnancy results in a hypothyroid condition in the offspring called endemic cretinism, and these individuals suffer from mental retardation, motor deficits, sensorineural deafness, and, even when hearing is not impaired, difficulties in language perception and usage (DeLong and Adams, 1992; Delange, 1992; Porterfield, 1994). Deafness and mental retardation are also characteristic of congenital hypothyroidism (Boyages and Halpern, 1993). Experimental perinatal hypothyroidism, in which circulating T4 was virtually eliminated by drug treatment [e.g., propylthiouracil (PTU) or methimazole], is associated with malformations of the organ of Corti and functional auditory deficits (Deol, 1973; Uziel *et al.*, 1980, 1981), as well as growth retardation, developmental delays in eye opening and weaning (Blake and Henning, 1985), hyperactivity (Tamasy *et al.*, 1986), and cognitive deficits (Davenport and Dorsey, 1972; Schalock *et al.*, 1979).

In a recent study, rats were exposed from Gestational Day (GD) 18 to Postnatal Day (PND) 21 to PTU at doses ranging from 1 to 25 ppm in the drinking water, and dose-dependent decreases in circulating thyroid hormones were observed in the offspring (Goldey *et al.*, 1995b). Auditory deficits were seen at 5 and 25 ppm PTU, but the 5 ppm dose produced auditory effects in the absence of other somatic and behavioral effects detected at the higher dose. These results indicated that the developing auditory system is particularly sensitive to reduced circulating thyroid hormones. Such findings suggest that auditory function may also be impaired by thyrotoxic environmental agents.

The current study describes the effects of developmental exposure to Aroclor 1254, a mixture of PCB congeners with an overall chlorine content of 54% (see Schulz *et al.*, 1989 for typical congener characterization), on circulating thyroid hormone concentrations and auditory function. Other somatic and behavioral endpoints which have been shown to be affected by developmental PCB exposure and/or hypothyroidism were also assessed. The results from the current study, together with those of a previous study (Goldey *et al.*, 1995b), support the hypothesis that some of the neurotoxic effects of developmental PCB exposure may be associated with alterations in thyroid function (Porterfield, 1994).

METHODS

Animals

Primiparous Long-Evans rats, obtained from Charles River Laboratory (Raleigh, NC) on GD 2 (the day of insemination was GD 0), were housed individually, in standard plastic hanging cages (45 × 24 × 20 cm) with sterilized pine shavings as bedding, in an AAALAC-approved animal facility. All experiments were approved in advance by the Health Effects Research Laboratory animal care committee of the U.S. Environmental Protection Agency. Animal rooms were maintained on a 12:12 hr photoperiod,

L:D (0600:1800), food (Purina Lab Chow) and tap water were provided *ad libitum*.

Beginning on GD 22, animals were checked twice daily (AM and PM) for births, and the date that birth was first discovered was assigned PND 0. On PND 4 (in cohort 1) or PND 6 (in cohort 2), litters were culled to 10 pups/litter, and 2 male and 2 female pups within each litter were given foot tattoos for individual identification (Avery and Spyker, 1977). Nontattooed pups were randomly selected from each litter for blood collection, and litter size was correspondingly reduced by 1 pup at each collection age. Animals were weaned on PND 21, and animals within a treatment group were housed in gender-matched pairs on PND 28.

Dosing

A commercial PCB mixture, Aroclor 1254 (AccuStandard, Inc.; Lot 6024), was administered to the dams daily via oral gavage from GD 6 to PND 21 (except for PND 1, when the dams were left undisturbed). Animals were exposed to Aroclor 1254 dissolved in corn oil at a dose volume of 1 ml/kg. In a preliminary investigation (cohort 1), 6 dams/treatment received 0 (corn oil), 1, 4, 10, or 40 mg/kg Aroclor 1254. Based on the findings from this first group of animals ($\geq 50\%$ pup mortality at the two highest doses), cohort 2 (10 dams/treatment) received 0, 1, 4, or 8 mg/kg Aroclor 1254.

Pup Growth and Development

All pups were born between 1300 hr on GD 22 (PND 0) and 0900 hr on GD 23 (PND 1). Pups in cohort 1 were weighed (by litter) on PND 1 and 4 and subsequent time points, whereas weighing of pups in cohort 2 did not begin until PND 6 in an effort to minimize any stress to the dam which may have contributed to early postnatal cannibalization of treated pups in cohort 1. Maternal body weights were recorded daily throughout the dosing period and general observations were made of the pups, including morbidity, cannibalization, and general appearance. Eye opening was monitored once daily beginning on PND 13. The ratio of pups within a litter with at least one eye open vs total pups was recorded each day until all pups' eyes were opened.

T4, T3, and Thyroid-Stimulating Hormone (TSH)

For cohort 1, blood samples were collected on PND 1, 7, 14, and 21 from two pups/litter/treatment. On PND 1, within-litter blood samples were pooled for analysis, whereas on PND 7–21, individual samples were assayed and the mean concentration per litter was used in the analysis. For cohort 2, samples were collected from one pup per litter on PND 7, 14, 21, 30, and 45. Samples were immediately placed on ice, the blood was allowed to clot for up to 2 hr, and centrifuged at 2500 rpm for 15 min. Serum samples were stored at -80°C until radioimmunoassay of total T3 (tT3) and T4 (tT4) from cohort 1 and 2, free T3 (fT3) and T4 (fT4) from cohort 2, and TSH from cohort 2. Standard assay kits were used for determination of free and total T3 and T4 concentrations (Diagnostic Products, Inc.; Los Angeles, CA). TSH was determined as described in Goldman *et al.* (1986, 1990) using materials kindly provided by the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases (iodination preparation I-7; reference preparation S-5). Individual tracers were radiolabeled with ^{125}I (New England Nuclear, Boston, MA), using chloramine-T (Greenwood *et al.*, 1963). The inter- and intraassay coefficients of variation were below 10% for all hormone assays.

Figure-Eight Maze Activity

One male and one female pup per litter were tested individually in one of 16 automated figure-eight mazes for 30 min on PND 13, 15, 17, 19, and 21, and for 1 hr on PND 30, 60, 90, and 120. Motor activity was detected by eight infrared photobeam pairs in each maze. Data were summated in 5-min intervals for analysis of within-session habituation (i.e., decrease in

activity within the test session) and as total counts per test session compared across test ages. A separate repeated measures analysis of variance (ANOVA) was performed on total counts/session across each of the two age blocks (i.e., across 30-min sessions for pups \leq PND 21 and across 1-hr sessions for animals \geq PND 30). For a more complete description of method, see Goldey *et al.* (1995b).

Acoustic Startle Response (ASR)

Habituation of ASR in weanlings. Habituation to the ASR was assessed in one tattooed male and female pup per litter on PND 24. Testing was conducted in eight sound-attenuated chambers as described previously (Goldey *et al.*, 1995b; for a more detailed description of ASR methodology, see Crofton, 1992). Briefly, each rat received a total of 50 trials (intertrial interval = 15 sec) in which each trial was initiated by a startle eliciting stimulus (120-dB, 40-msec white noise burst) and response data were collected for 64 msec. Response amplitude, taken from each animal's average response curve, was calculated across trials within a block. Ten sequential blocks of 5 trials were used to assess habituation (decreased response amplitude across trials) to the eliciting stimulus. The mean response amplitude across all trial blocks was also calculated for treatment comparisons.

Reflex modification audiometry in adult offspring. Auditory thresholds were determined beginning on PND 85 utilizing reflex modification audiometry (for complete description of method, see Goldey *et al.*, 1995b). One male and one female rat per litter were evaluated from each exposure group. Each rat was placed in a test cage and received a total of 240 trials. Each trial consisted of an invariable eliciting stimulus (S2; 120 dB SPL, 40 msec burst of broadband white noise), preceded by a prepulse tone (S1) at one of five frequencies (1, 4, 16, 32, or 40 kHz). The trials were arranged in 10 blocks, with 24 trials per block. Each block contained a blank control trial, during which only S2 was presented, and 23 trials in which a different intensity (-6 to 90 dB SPL, in increments of 3 or 6 dB) of the S1 stimulus (40-msec duration, 2.5-msec rise/fall) was presented 90 msec prior to the S2 stimulus. Auditory thresholds were defined as the S1 intensity above which the response to S2 was inhibited (see Crofton, 1992). Animals were tested for one S1 frequency per day, with the five S1 frequencies randomly assigned across test days. The startle response amplitude was also calculated as the mean response amplitude across all blank control trials.

Data Analysis

ANOVA procedures were used for all main and simple effects tests (SAS, 1989). In the case of more than one independent variable, significant interactions were followed by simple-effects ANOVA tests for each independent variable. Repeated measures ANOVAs (multivariate) were used where appropriate [i.e. to compare body weights and activity counts (across age), acoustic startle habituation (across trial block), and auditory thresholds and startle amplitudes (across the five S1 frequencies)]. Gender was a within-litter repeated measure (to control for possible "litter effects"), whereas treatment was a between-litter factor for all variables. For each significant effect of treatment, mean contrast comparisons were made using Duncan's new multiple range test. An alpha value of 0.05 was used for all comparisons. The more credible statistical interpretation comes from the second, larger cohort of animals, although we have noted the similarity of effects between the cohorts to emphasize the replicability of our findings.

RESULTS

Maternal Effects

In cohort 1, the 40 mg/kg dose of Aroclor 1254 was acutely toxic to the pregnant dams, and after 1 week of dosing, these animals stopped eating and failed to gain weight. Dosing was discontinued in this group after 10 days

(GD 16). Only four of the six rats in this group were ever pregnant; two dams resorbed all fetuses (11 and 14 fetuses, respectively), and the remaining two dams gave birth to either dead pups or pups which died within 24 hr of birth. Remaining dams from cohort 1 (control, 1, 4, or 10 mg/kg) were necropsied when pups were weaned, and no fetuses had been resorbed for any of these treatment groups. Based on these findings, no necropsy was performed on dams from cohort 2. In addition, no overt maternal toxicity (no weight gain deficits or mortality) was evident at doses ≤ 10 mg/kg in cohorts 1 or 2.

Offspring Development

Aroclor 1254 did not affect the length of gestation, and PCB-induced body weight deficits in offspring were similar between cohorts 1 and 2. In cohort 2, deficits in pup body weight were apparent on PND 6 in the 4 and 8 mg/kg groups, and these deficits became more pronounced throughout the preweaning period (Fig. 1, left). These findings were supported by a significant effect of treatment [$F(3,36) = 16.9$, $p < 0.05$] and a significant age by treatment interaction [$F(12,144) = 12.06$, $p < 0.05$]. Body weight deficits persisted into adulthood (Fig. 1, right); findings which were supported by a significant effect of treatment [$F(3,31) = 10.2$; $p < 0.05$]. Step-down ANOVAs revealed effects at all ages \geq PND 6 [all $F(3,36) \geq 7.83$; $p < 0.05$]. There were no significant interactions of treatment with age or gender. In cohort 1, only pups from the 10 mg/kg group showed body weight deficits. On PND 1, body weight was 10% below controls (controls = $6.5 \text{ g} \pm 0.24$), and severe deficits (near 30%) were observed from PND 4 through PND 13. Thereafter, the deficits in this treatment group were more moderate (approx 15%) and were no longer statistically different from controls (most likely due to the fact that the most severely affected individuals at this exposure level died prior to PND 16). Findings in cohort 1 were supported by a significant effect of treatment [$F(3,17) = 6.44$; $p < 0.05$] and a significant age by treatment interaction [$F(3,18) = 2.4$, $p < 0.05$] followed by step-down ANOVAs at each age ≤ 13 [all $F(3,17) \geq 4.28$; $p < 0.05$].

Eye opening was earlier in pups from the 8 mg/kg group compared to controls in cohort 2 (Fig. 2). Approximately 25% of pups in the 8 mg/kg group had at least one eye open by PND 14, whereas no control pups' eyes were open at this age. By PND 15, 80% of high-dose pups, compared to 50% of controls, had their eyes open. These findings are supported by a significant interaction between age and treatment [$F(12,144) = 3.30$; $p < 0.05$] and significant effects of treatment on PND 14 and PND 15 [$F(3,36) \geq 3.42$; $p < 0.05$]. The value for litters in the 8 mg/kg group was significantly different from the control value at these two ages. A comparable, though nonsignificant (perhaps due to the small sample size), trend toward early eye opening was seen in pups from the 10 mg/kg group in cohort 1.

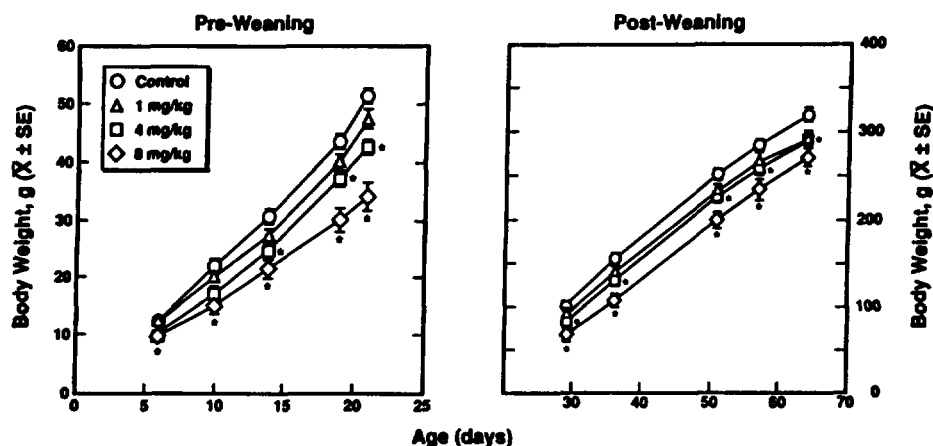


FIG. 1. Body weights (mean \pm SE) of pups from the 4 and 8 mg/kg Aroclor 1254-exposed groups were significantly reduced compared to controls by PND 6, and these effects were apparent throughout the preweaning period (left). Weight gain was not significantly reduced in preweanlings at the 1 mg/kg dose. The effects on body weight were persistent into adulthood (right). $N = 8-11$ litters per treatment. *Significantly different from the control value at the same age ($p < 0.05$).

In cohorts 1 and 2, offspring mortality was high in the highest treatment groups, i.e., 58% of pups died by PND 21 in the 10 mg/kg group, whereas in the 8 mg/kg group, 25% of the pups had died by PND 12 and 50% by PND 21. In contrast, by PND 21 the controls, 1 and 4 mg/kg groups had only 3, 5, and 15% mortality, respectively. Cannibalism by the dam was common in the highest treatment groups from both cohorts (not quantified). Approximately 10 pups from the 8 mg/kg group were notably jaundiced (personal observation) before they died (or were cannibalized). Others became weak, began gasping, and their bellies became distended

with air prior to death. Due to the potential for compromised immune function in PCB-treated animals (Harper *et al.*, 1993; Silkworth and Grabstein, 1982), two treated dams and 6 of the dying pups (from four litters) were killed on PND 12 and their tissues subjected to a screen for a number of potential rat pathogens, including Kilham rat virus (Pathology Associates, Research Triangle Park, NC). Blood samples, drawn from sentinel animals within the colony, were also analyzed. No pathogens were identified in tissues collected from pups or sentinels.

Total T4 and T3, Free T4 and T3, and TSH Concentrations

Effects of Aroclor 1254 on serum concentrations of T4, T3, and TSH were similar for male and female offspring. In cohort 1, a dose-dependent reduction in tT4 concentration was apparent on PND 1, 7, 14, and 21 [all $F(3,9-18) \geq 5.4$; $p < 0.05$, Table 1]. However, it should be noted that on PND 1, even control samples had tT4 concentrations which were below the lowest point on the standard curve used in the assay. For cohort 2, tT4 levels were similarly reduced in a dose-dependent manner on PND 7, 14, and 21, in all of the Aroclor 1254 treatment groups compared to controls (Fig. 3, top left). These findings are supported by a significant treatment by age interaction [$F(12,133) = 89.4$; $p < 0.05$], significant treatment effects at each age ≤ 30 [all $F(3,24-31) > 18$; $p < 0.05$], and significant mean contrast comparisons between the controls and each treatment at particular ages as shown in Fig. 3 ($p < 0.05$). By PND 30, the tT4 concentration in the 1 mg/kg group had returned to control levels, and by PND 45, the tT4 concentrations in the 4 and 8 mg/kg groups had also recovered to near control levels.

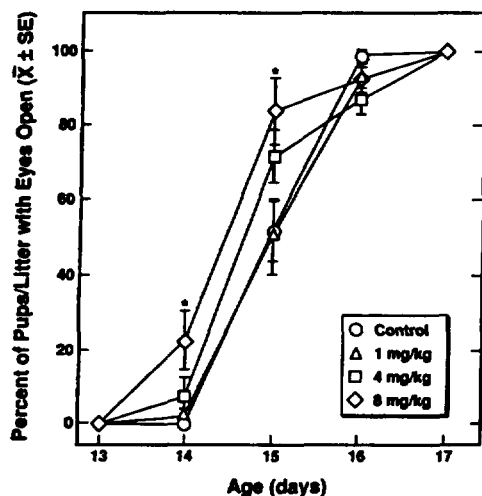


FIG. 2. Age of eye opening was determined as the percentage of pups within a litter ($N = 8-11$ litters per treatment) with at least one eye open at each age (mean percentage \pm SE). Asterisks indicate that the value for litters in the 8 mg/kg group was significantly different from the control value at the same age ($p < 0.05$).

TABLE 1

Cohort 1: Total Serum Thyroxine (T4) and Triiodothyronine (T3) Concentrations from Offspring of Rats Exposed to Aroclor 1254

Age (days)	Control	1 mg/kg	4 mg/kg	10 mg/kg
Total serum T4 concentration, ng/ml (mean \pm SE)				
1	5.26 \pm 0.55* (5)*	4.38 \pm 1.20 (3)	1.78 \pm 0.61* (3)	1.90 \pm 0.30* (2)
7	28.4 \pm 2.26 (6)	14.2 \pm 2.04* (6)	5.74 \pm 0.64* (5)	2.71 \pm 0.70* (4)
14	68.6 \pm 1.90 (6)	35.1 \pm 3.03* (6)	13.44 \pm 3.09* (5)	5.61 \pm 0.76* (5)
21	51.4 \pm 4.84 (4)	19.7 \pm 2.85* (5)	6.58 \pm 1.50* (5)	NC
Total serum T3 concentration, ng/ml (mean \pm SE)				
1	0.116 \pm 0.027*	0.103 \pm 0.025	0.170 \pm 0.032	0.040 \pm 0.040
7	0.467 \pm 0.049	0.381 \pm 0.026	0.411 \pm 0.035	0.520 \pm 0.044
14	0.995 \pm 0.047	0.900 \pm 0.067	0.767 \pm 0.052*	0.785 \pm 0.055*
21	1.211 \pm 0.046	1.176 \pm 0.075	0.995 \pm 0.052*	NC

Note. NC, serum not collected due to insufficient number of animals remaining from the 10 mg/kg dose at this age.

* Values for T4 and T3 on PND 1 were below the lowest value on the standard curve for each assay (10 and 0.2 ng/ml, respectively).

* Number of litters sampled (one or two pups/litter) are indicated in parentheses (same for T3). If blood from two pups/litter was assayed, an average value per litter was calculated and used in the statistical analysis.

* Values were significantly different from age-matched control values ($p < 0.05$).

The effects of Aroclor 1254 on serum fT4 were similar to those on tT4 (Fig. 3, bottom left). The only difference was that on PND 45, fT4 levels were still significantly reduced in the 4 and 8 mg/kg groups. The fT4 findings

were supported by a significant treatment by age interaction [$F(12,133) = 3.82$; $p < 0.05$], significant treatment effects at each age [all $F(3,24-31) > 4$; $p < 0.05$], and significant mean contrast comparisons between the con-

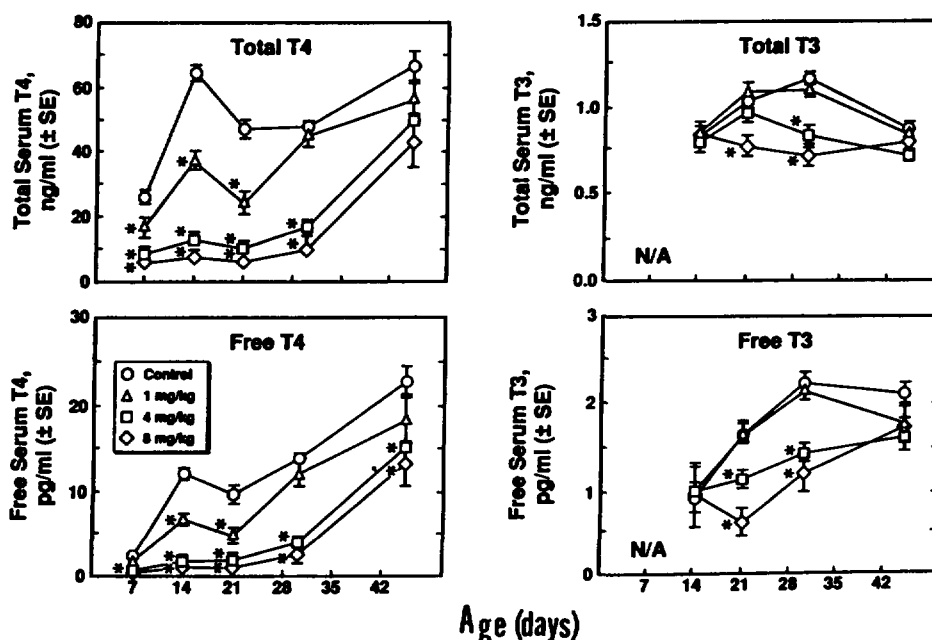


FIG. 3. Aroclor 1254 exposure dramatically lowered total serum T4 (top, left) concentration in the 4 and 8 mg/kg groups at all ages sampled until PND 45, whereas effects at the 1 mg/kg dose were attenuated by PND 30. The effects on free T4 (bottom, left) mimicked those of total T4. For free T4, the only nonsignificant differences were between the controls and 1 mg/kg group on PND 30 and 45. Total T3 was not affected at any dose on PND 14, whereas total T3 was significantly reduced in the 8 mg/kg group on PND 21 and 30, and the 4 mg/kg group on PND 30 (top, right). Free T3 was significantly reduced in the 4 and 8 mg/kg groups on PND 21 and 30 (bottom, right). $N = 8$ serum samples per time point. *Significantly different from the control values at the same age ($p < 0.05$).

TABLE 2
Thyroid-Stimulating Hormone (TSH) Concentrations in Serum from Offspring of Rats Exposed to Aroclor 1254

Age (days)	TSH concentration, ng/ml (mean \pm SE) ^a			
	Control	1 mg/kg	4 mg/kg	8 mg/kg
7	1.16 \pm 0.12	1.21 \pm 0.08	NA	NA
14	1.73 \pm 0.15	1.87 \pm 0.16	1.64 \pm 0.25	2.16 \pm 0.38
21	1.31 \pm 0.02	1.94 \pm 0.32	1.90 \pm 0.25	1.50 \pm 0.25
30	1.51 \pm 0.18	1.73 \pm 0.25	2.44 \pm 0.31	2.98 \pm 0.89
45	1.72 \pm 0.33	1.73 \pm 0.17	2.00 \pm 0.27	2.77 \pm 0.46

^a There were no statistically significant differences in TSH concentrations between controls and treated pups at any age. Serum samples were from one pup from each of 8 to 10 litters/treatment. NA, not assayed due to insufficient samples volume from animals at these dosages.

trols and each treatment group ($p < 0.05$) at each sampled age.

In general, the effects of Aroclor 1254 on serum T3 concentrations were more moderate than the effects on T4. No significant effects of treatment on tT3 were seen for cohort 1 (Table 1). However, in cohort 2, tT3 concentration was significantly reduced on PND 21 and 30 in the 8 mg/kg group, whereas in the 4 mg/kg group, tT3 reductions were detected only on PND 30. No effects on tT3 were seen on PND 14, and subsequent effects were attenuated by PND 45 (Fig. 3; top right). These findings are supported by a significant treatment by age interaction [$F(9,99) = 3.85$; $p < 0.05$], significant effects of treatment on PND 21 and 30 [$F(3,24 \text{ or } 27) > 6$; $p < 0.05$], and significant mean contrast comparisons on PND 21 and 30 ($p < 0.05$). It may be particularly important that fT3 was significantly reduced in the 4 and 8 mg/kg treatments on PND 21 and 30 (Fig. 3; bottom right).

There were no statistically significant changes in circulating TSH concentrations at any age sampled, although the dose-dependent trend in the data suggests that TSH may be increased in some PCB-exposed offspring (Table 2).

Figure-Eight Maze Activity

Male offspring exposed to 4 or 8 mg/kg showed a transient reduction in activity on PND 15 compared to controls (Fig. 4, left). This finding is based on a significant threeway interaction of gender by age by treatment [$F(12,66) = 3.69$; $p < 0.05$], a significant gender by treatment interaction [$F(3,28) = 3.33$; $p < 0.05$], and for males, a significant age by treatment interaction [$F(12,112) = 2.99$; $p < 0.05$], a significant effect of treatment on PND 15 [$F(3,31) = 6.85$; $p < 0.05$], and significant mean contrast comparisons on PND 15 for the 4 and 8 mg/kg groups compared to controls ($p < 0.05$). Although females from these treatment groups also showed lower activity counts compared to controls on PND 15, the effect in females was not significant (Fig. 4; right). It should be noted that the variance around the mean

for control females in this study is unusually high compared to historical controls within our laboratory (Crofton and Goldey, personal observation). In addition, we recently tested a subsequent cohort of animals similarly exposed to 8 mg/kg Aroclor 1254 and found that treated animals of both sexes showed significantly lowered activity on PND 15 compared to controls (Crofton and Goldey, unpublished data).

ASR

Pups exposed to 8 mg/kg Aroclor 1254 showed reduced startle amplitudes across all trial blocks when animals were tested on PND 24 (Fig. 5). These findings are supported by a significant effect of treatment [$F(3,28) = 8.87$; $p < 0.05$]. There were no significant interactions with gender or trial block. Startle amplitudes decreased (habituated) over the 50-trial session, a finding supported by a significant main effect of trial block [$F(9,20) = 16.4$; $p < 0.05$]. Based on mean contrast comparisons, the 8 mg/kg group was significantly different from the control group during all trial blocks ($p < 0.05$). Startle amplitudes, though reduced on PND 24 (Fig. 6, left), had recovered to control levels when animals were tested as adults (Fig. 6, right).

Reflex modification audiometry in adult offspring. Auditory thresholds were elevated at the 1-kHz frequency (in cohorts 1 and 2) for animals exposed to ≥ 4 mg/kg (Fig. 7; see inset for cohort 1). There were no significant auditory threshold deficits at higher frequencies (4, 16, 32, or 40 kHz). The effects on auditory thresholds were similar in males and females. These effects were supported by a significant frequency by treatment interaction [$F(12,74) = 2.37$; $p < 0.05$], an overall effect of treatment [$F(3,31) = 4.79$; $p < 0.05$], a significant effect of Aroclor 1254 treatment at 1 kHz [$F(3,34) = 11.72$; $p < 0.05$], and significant mean contrast comparisons between the controls and both the 4 and 8 mg/kg groups at 1 kHz.

DISCUSSION

The disruptive effect of Aroclor 1254 on auditory function represents a novel finding in the characterization of PCB-

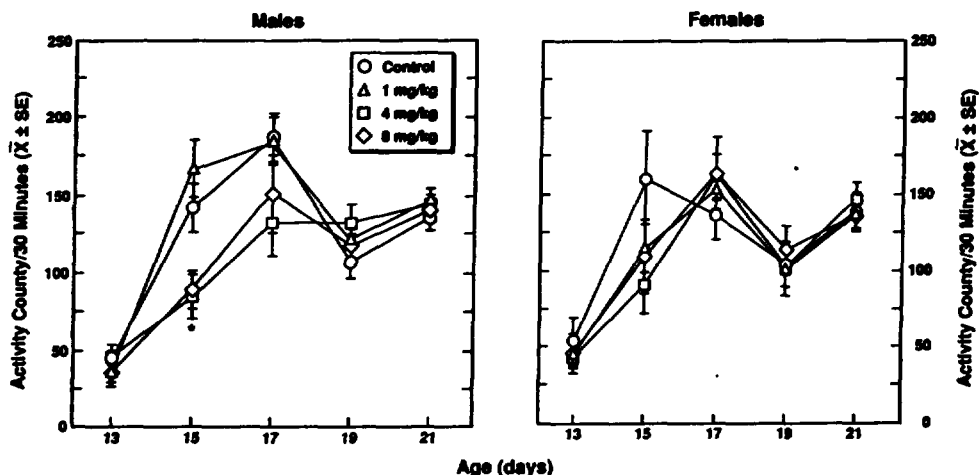


FIG. 4. Aroclor 1254 exposure caused a transient reductions and/or developmental delay in figure-eight maze activity (mean \pm SE) in preweanling male rats (left). This effect was apparent in the 4 and 8 mg/kg males on PND 15 (indicated by asterisk). Female pups did not appear to be affected by Aroclor 1254 exposure, although this apparent gender-dependent effect should be viewed with some uncertainty (see text for explanation). $N = 8$ litters per treatment (one male and one female/litter).

induced developmental neurotoxicity, and the present results suggest a link between the observed ototoxicity and reduced thyroid hormone concentrations. Hypothyroidism occurring during auditory system development causes profound morphological abnormalities of the cochlea and functional auditory deficits (Deol, 1973; Uziel *et al.*, 1980, 1981). The human cochlea develops prenatally (Pujol *et al.*, 1990), whereas the rat cochlea develops postnatally (Rubel, 1978; Müller, 1991b); therefore, auditory damage results when hypothyroidism occurs during the second trimester in humans (DeLong, 1993; Boyages and Halpern, 1993; Boyages, 1993) or during the early postnatal period in rats (Uziel, 1985).

Work with rats exposed to propylthiouracil has shown that the severity of auditory dysfunction is related to the degree of thyroid hormone suppression during the preweaning period (Goldey *et al.*, 1995b). Specifically, severe auditory threshold shifts (>50 dB) were caused by PTU dosages (25 ppm) which induced profound reductions in circulating levels of thyroid hormones, whereas more moderate reductions in hormone levels (5 ppm PTU) caused 20- to 30-dB shifts in auditory thresholds (Goldey *et al.*, 1995b). In the present study, Aroclor 1254 exposure caused a 20- to 30-dB shift in low-frequency auditory thresholds and changes in hormone levels which were similar to those induced by an intermediate dosage of PTU (5 ppm). The following discussion may provide greater understanding of the ototoxicity observed in Aroclor 1254-exposed animals by relating the magnitude and timing of thyrotoxic effects of Aroclor 1254 and PTU with the ontogeny of the cochlea.

Whereas previous studies have noted effects of Aroclor 1254 on thyroid function (Collins and Capen, 1980; Morse and Brouwer, 1994), the current results provide a dose-response assessment and ontological profile of the thyrotoxic effects of exposure to this PCB mixture. Offspring of dams exposed to ≥ 4 mg/kg Aroclor 1254 showed deficits in tT4 and fT4 concentrations (80–90% reduction from control levels) throughout the postnatal period until recovery to near normal levels by PND 45, and these effects on T4 were similar in magnitude to those seen with intermediate dosages (5 ppm) of propylthiouracil (Goldey *et al.*, 1995b). Deficits in tT4 were also seen at the lowest dose of Aroclor 1254 (1 mg/kg), although the effects at this dose were more moderate (50% below controls) and were attenuated sooner (by PND 30). Offspring in this lowest treatment group did not reveal any physical or functional effects on the other endpoints

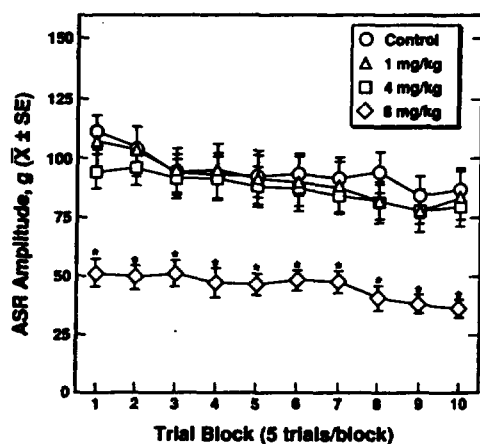


FIG. 5. Acoustic startle habituation testing on PND 24 revealed that animals exposed to 8 mg/kg of Aroclor 1254 showed reduced amplitudes across all trial blocks (five trials/block). $N = 8$ litters per treatment (one male and one female/litter). Asterisks indicate that the value was significantly different from the control value during a particular trial block ($p < 0.05$).

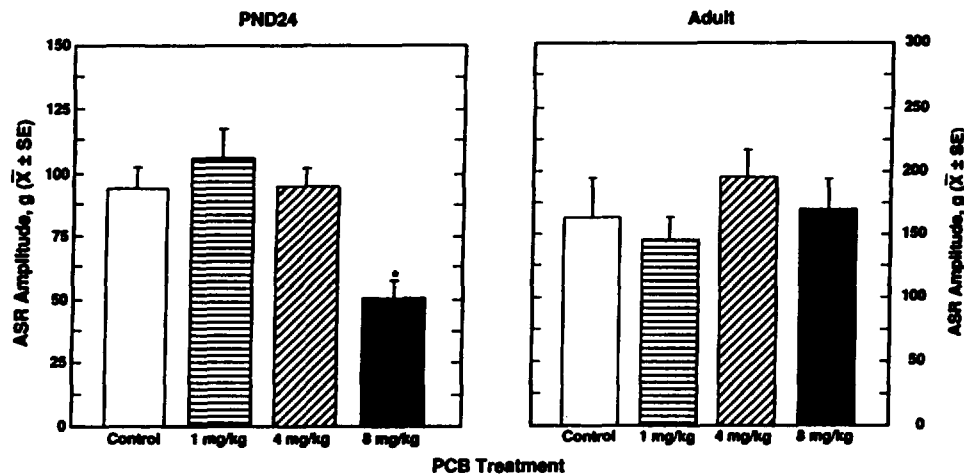


FIG. 6. Acoustic startle response amplitudes (mean \pm SE), reduced in pups from the 8 mg/kg dose on PND 24 (left), were not affected when animals were tested as adults (right). *Significantly different from the age-matched control value. $N = 8$ litters per treatment (one male and one female/litter).

measured, indicating that the animals in this group overcame and/or compensated for the reduced levels of serum T4 and/or for any direct toxicity of the PCBs. Whereas a previous report suggests that circulating fT4 is more reduced in female offspring exposed to Aroclor 1254 compared to males (Morse and Brouwer, 1994), we found no gender-dependent

effects of Aroclor 1254 on thyroid hormones at any age sampled. It is possible that the different results between the studies may be due to the differences in the exposure paradigms utilized (Morse and Brouwer exposed Wistar rats from GD 10 to 16 to 0, 5, or 25 mg/kg Aroclor 1254).

The biologically active form of thyroid hormone, T3, is produced by the deiodination of T4 (Edmonds, 1987). Significant effects of Aroclor 1254 on fT3 and tT3 were not detected until PND 21, and the age of onset of effects of Aroclor 1254 on T3 was notably similar to that for intermediate dosages (5 ppm) of PTU (Goldey *et al.*, 1995b). The results of these two studies indicate that pronounced deficits in circulating T4 concentrations do not necessarily cause a concomitant drop in circulating T3 concentration, and that prolonged T4 reduction may be necessary to reduce circulating T3 concentrations.

An estimated 80% of the thyroid hormone supply utilized by the brain comes from circulating T4 which is transported to the brain by thyroxine-binding prealbumin (transthyretin) and subsequently deiodinated intracellularly to T3 by 5'-D-II deiodinase (Edmonds, 1987). Concentrations of 5'-D-II deiodinase increase in response to lowered T4 levels, thereby protecting central structures against decreased nominal hormone concentrations. In the periphery, cells may take up T3 directly from the circulation and/or utilize the 5'-D-I deiodinase, which is not upregulated when T4 levels fall (Edmonds, 1987). Therefore, if the cochlea relies on 5'-D-I, it and other peripheral structures may be at greater risk from the effects of decreased serum thyroid hormone concentrations than centrally located nervous system structures (which utilize 5'-D-II). Actual determination of which enzyme is active in the cochlea would shed further light on this possibility.

Cochlear development proceeds in a basal to apical direc-

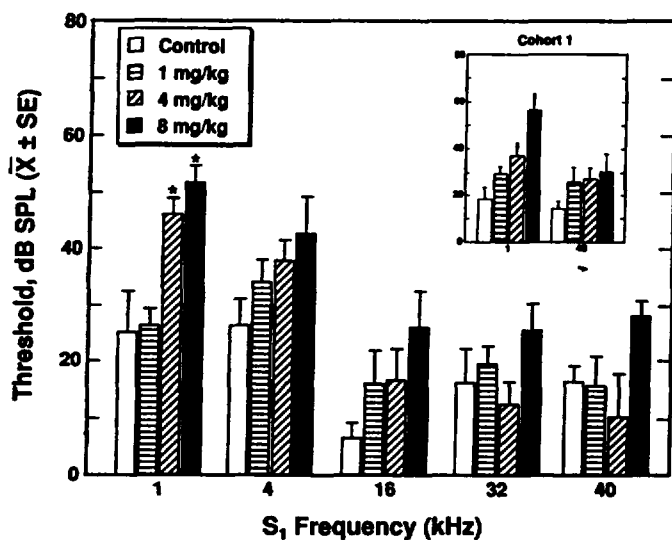


FIG. 7. Auditory thresholds at 1 kHz were significantly higher for animals exposed to 4 or 8 mg/kg Aroclor 1254 compared to controls in cohort 2. Auditory thresholds at frequencies ≥ 4 kHz were not significantly affected by Aroclor 1254 exposure. (Inset) in cohort 1, only two frequencies were tested (1 and 40 kHz), and offspring exposed to 4 or 10 mg/kg Aroclor 1254 showed significantly higher auditory thresholds than controls for a 1-kHz tone (in inset, the black bar represents results from animals exposed to 10 mg/kg Aroclor 1254). $N = 8$ litters per treatment (cohort 2) or 5 or 6 litters per treatment (cohort 1). One male and one female/litter were tested. *Significantly different from the control value ($p < 0.05$).

tion, and regions ultimately sensitive to lower frequencies mature later than higher frequency regions (Rubel, 1978; Puel and Uziel, 1987; Müller, 1991a,b). In the rat, Aroclor 1254 exposure may have preferentially affected low-frequency thresholds because circulating T3 concentrations were not reduced until sometime after the second postnatal week, a period when apical regions of the cochlea are still maturing (Müller, 1991b), and thus may be more susceptible to disruption than more developed regions (Eggermont, 1986). Our findings further suggest that fT3 may be more responsible than tT3 for the observed effects because fT3 was reduced in both the 4 and the 8 mg/kg groups by PND 21, whereas tT3 was only reduced in the highest treatment group by this age.

Whereas Aroclor 1254 exposure resulted in a low frequency, auditory threshold shift, PTU caused threshold shifts across a broad spectrum of frequencies. The widely differing mechanisms of action between PTU and Aroclor 1254 are likely to contribute to the differences in frequency specificity. For example, unlike PTU, the structural similarity of some PCB congeners, and/or their metabolites, may allow them to directly bind to (and thus block) thyroid hormone receptors (McKinney, 1989), a consequence which could exacerbate effects on sensitive targets when T3 levels fall to a point where the hormone cannot compete effectively for the receptor sites.

The lack of effect of maternal exposure to Aroclor 1254 on TSH concentrations, despite sharply reduced circulating thyroid hormone concentrations, is consistent with recent reports (Morse and Brouwer, 1994). If, as previously indicated (Rickenbacher *et al.*, 1986; McKinney and Waller, 1994), PCB congeners and/or their metabolites mimic thyroid hormones, they may bind to thyroid hormone receptors in the pituitary and block TSH release (Morse and Brouwer, 1994), thus inhibiting one mechanism which would provide compensation for reduced hormone levels. While the activity of 5'-D-II in the brain has been shown to increase in response to PCB-induced reductions in circulating T4 levels (Morse *et al.*, 1993), these workers have demonstrated that PCBs cause reduced brain levels of T4 and, to a lesser extent, T3, during development (Morse and Brouwer, 1994). This indicates that compensatory increases in 5'-D-II activity, while helping to maintain hormone homeostasis, may not completely protect the brain from PCB-induced reduction in thyroid hormone concentrations.

In addition to effects on auditory and thyroid function, Aroclor 1254 also affected motor activity development and growth of the exposed offspring. Offspring exposed to 8 mg/kg Aroclor 1254 showed a deficit and/or delay in activity on PND 15, a finding which was similar in timing, direction and magnitude to that of offspring from the intermediate dosage group in the PTU study (Goldey *et al.*, 1995b), suggesting that the observed effect may be exacerbated by hypo-

thyroidism. Similarly, transient deficits in motor activity were noted for 14 day old offspring maternally exposed to Fenclor 42 (Pantaleoni *et al.*, 1988). In general, the reported effects of developmental PCB exposure on motor activity are varied and appear to be dependent on such factors as species, exposure regimen, test method and age of evaluation (Tilson *et al.*, 1990). It is interesting to note that children exposed to high levels of PCBs and related compounds demonstrate mildly increased activity levels; an effect which appears to persist throughout childhood (Chen *et al.*, 1994).

Growth deficits in pups exposed to ≥ 4 mg/kg, and high offspring mortality at ≥ 8 mg/kg, indicated that Aroclor 1254 is acutely toxic to the developing rat at dosages which produce no maternal body weight loss or mortality. These findings are in general agreement with those of Overmann *et al.* (1987). The mortality in PCB-treated pups may be more related to PCB-induced liver toxicity (WHO, 1993) than thyrotoxicity, as some of the dying pups appeared jaundiced prior to death.

In light of the severe delay in eye opening in pharmacologically hypothyroid animals (Goldey *et al.*, 1995b), early eye opening in the high dose animals exposed to Aroclor 1254 (8 mg/kg in cohort 2 and 10 mg/kg in cohort 1) was an unexpected finding. This effect cannot be attributed to classical effects of hypothyroidism, and it provides further evidence that Aroclor 1254 may have a number of different mechanisms of toxic action in the developing rat. In contrast to our findings, Overmann *et al.* (1987) reported no effect of maternal Aroclor 1254 exposure on the age of eye opening. However, the highest dosage group (269 ppm in diet) in the Overmann *et al.* study did not survive past PND 7, and the body weight deficits and pup mortality were low in their next highest treatment group (26 ppm) relative to the highest dose used in the present study (8 mg/kg, po). This comparison suggests that the highest dosage used in the present study resulted in an exposure level which fell between the two highest dosages used by Overmann *et al.* (1987). It also suggests that early eye opening due to Aroclor 1254 exposure may be limited to higher exposure levels. However, because eye opening is typically evaluated only once per day, and is further confounded by imprecise knowledge of the exact moment of conception, subtle "low-dose" changes in age of eye opening may be difficult to discern. It is interesting to note that a single exposure of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to pregnant dams on GD 15 induced early eye opening in rat pups (Mably *et al.*, 1992). Therefore, careful attention to assessment of this endpoint may yield important information on the developmental effects of PCBs and related compounds.

The development of the auditory startle response is sensitive to thyroid hormone disruption (Schneider and Golden, 1987) and previous investigators have noted a delayed onset of the response in rat pups maternally exposed to Aroclor

1254 (Overmann *et al.*, 1987). In the present study, the response was only assessed on PND 24, and pups from the 8 mg/kg group (and in the 10 mg/kg group in cohort 1) showed reduced startle response amplitudes at this age. These findings may relate to a delay in the maturation of the response, possibly caused by hypothyroidism. This hypothesis is supported by the overt effect of PTU on the response in 24 day old pups (Goldey *et al.*, 1995b). However, further characterization of the response (e.g., assessment of the onset and maturation of the reflex) is needed to strengthen our understanding of the role of reduction in thyroid hormone concentrations on the development of this reflex.

In summary, our results indicate that developmental Aroclor 1254 exposure caused a transient delay in the development of motor activity and long-lasting hearing deficits. While PCBs may have a direct effect on the developing substrates which control these functions, an indirect effect on these endpoints, via PCB-induced reduction in circulating thyroid hormones, is suggested. This latter explanation is further supported by recent findings that the effects of developmental Aroclor 1254 exposure on motor activity and auditory thresholds were significantly attenuated by postnatal thyroxine "replacement" therapy (Goldey *et al.*, 1995a).

It has been hypothesized that PCBs produce reduced thyroid hormone concentrations in humans (McKinney and Pedersen, 1987), and Porterfield (1994) recently argued that PCB exposure in humans may result in maternal and fetal thyroid dysfunction which may, in turn, affect the developing brain. Importantly, a recent study of pregnant women and their infants documents a positive correlation between the concentration of PCBs and related compounds in breast milk and reduced maternal and neonatal thyroid hormone concentrations (Koopman-Elseboom *et al.*, 1994). Other epidemiological studies have indicated that children exposed to PCBs demonstrate behavioral problems (Huisman *et al.*, 1995), including cognitive deficits (for review, see Seegal and Schantz, 1994), effects which may be exacerbated by auditory deficits.

The results of the current study broaden our understanding of the potential human health risks associated with PCB exposure and imply that the developing auditory system may be a particularly sensitive target for the direct or indirect (e.g., hypothyroid) effects of PCBs. Although not directly related to effects on audition, the "yucheng" children (children exposed to high concentrations of PCBs and dibenzofurans due to maternal ingestion of contaminated cooking oil; see Hsu *et al.*, 1985) have shown a prevalence of middle ear disease (Chao and Hsu, 1994) and neurophysiological changes in centrally mediated auditory event-related potentials, P300 (Chen and Hsu, 1994). Therefore, further experimental and epidemiological investigations of the hypothesis that auditory deficits are related to PCB-induced thyrotoxicity seem warranted.

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